KORESPONDENSI PAPER

JUDUL : Application of Bioaugmentation on Ammonia in Sediment of Culture medium of Tiger Shrimps (*Penaeus Monodon*) In Differents Salinities

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1	Submission Acknowledgement	6 July 2009	Email from Ocky Karna Radjasa: • Initial manuscript • Blind reviewed	2-8
2	Submission Acknowledgement	14 September 2009	Email from Ocky Karna Radjasa: • Manuscript Accepted	10-15

Submission acknowledgement

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Dear Sarjito

This to acknowledge the submission of a manuscript to Journal of Coastal Development with a title of Application of Bioaugmentation on Ammonia in Sediment of Culture medium of Tiger Shrimps (Penaeus monodon). In differents salinities
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Thank you for submitting you work to Journal of Coastal Development.

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INITIAL MANUSCRIPT

APPLICATION OF BIOAUGMENTATION TO SOLVE AMMONIA INTHE SEDIMENT OF THE CULTURE MEDIUM OF TIGER SHRIMP (*Penaeus monodon* F.) IN DIFFERENT SALINITIES

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Abstract

Bioaugmentation was used to solve the ammonia problem, because this method is relatively safe to the environment. The objective of this research was to determine the influence and effectiveness of bioaugmentation on the reduction of ammonia in the sediment in culture medium of Tiger shrimp (P. monodon). Laboratory scale experiment and splitted plot randomized design was performed. The main treatment was probiotic epicin concentration of 0; 0.5; 1.0; and 1 ppm; as sub-treatment was the salinity (20; 25 and 30 ppt), and as a group was dayobservation (0; 2; 4 and 6). Sediment from intensive culture system was taken from brackish water pond of Brackishwater Aquaculture Development Project (BADP), Jepara. Sediment ammonia was analyzed using methods of Parson et al.,(1989). The research was conducted at hatchery of Fisheries and Marine Science Faculty of Diponegoro University, Teluk Awur, Jepara. The result indicated that bioaugmentation was able to reduce ammonia concentration in the sediment. Epicin has high significantly effected on the reduction of ammonia in the sediment ($p<0.01$). The capability of epicin as a bioaugmentation started on the day of 2. Therefore, the results also showed that a time (day) was highly significant affect on the reduction of ammonia in the sediment ($p<0.01$). However, salinity did not affect on the reduction of ammonia in the sediment ($p>0.05$). The most effective epicin dose to reduce ammonia in the sediment of the culture medium of Tiger shrimp (P. monodon) was 1.5 ppm.

Key words: Bioaugmentation, Ammonia, Epicin, Sediment, Salinity

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INTRODUCTION

The problem of high pollutant concentration such as uneaten feed and waste products in sediment has been frequently faced at the penaid shrimp (*Penaus monodon* F.) cultureand, in turn, it may reduce the water quality (Maguire and Allan, 1990). The increasing of pollutant or toxic substance in both sediment and water lead to a limitatian of the life process and in certain condition, it may increase the shrimp mortality (Apud, 1989; Maguire and Allan, 1990). Therefore, toxic substance, including ammonia, has caused the main failure of the culture (Maguire and Allan, 1990) and one of limiting factors in the intensive aquaculture system (Forteath, 1988).

Advanced technology in aquaculture for both semi and intensive shrimp culture in turn it needs intensive feeding (Apud, 1989). Inappropriate feeding caused increasing of the organic matter waste in the brockish wish pond sediment (Maguire and Allan, 1990). Decomposition of the organic matter, in the sediment, produce ammonia that is toxic for the shrimps (Boyd, 1989; Colt and Amstrong, 1991; Maguire and Allan, 1990). Besides that, shrimps as an amonotelic organism secrete ammonia through the gills about 60–80%. It may influence the ammonia concentration in the water (Claybrook,1993).

To solve the decreasing of the quality of tiger shrimp culture medium due to the increasing ammonia concentration, biological methods (bioaugmentation) could be done (Kinne, 1976; Liong and Subraniam, 1996). One of the bioaugmentation was conducted using probiotic “epicin” that contains bacterial decomposer (Briggs and Turnbull, 1995; Kinne, 1976; Sarjito *et al*., 1999). The methods was more effective compared to both mechanical and chemical methods (Kinne, 1976) and save to the environment (Liong and Subraniam, 1996).

Bioaugementation has been used inshrimp culture, however the study about it is very limited (Liong and Subraniam, 1996). Bioaugmentation is biotechnology applied in aquaculture by adding both nitrification anddecomposer bacteria in the water (Liong and Subraniam, 1996; Sarjito *et al*., 1999) in order to enhance aquaculture production and to save the environment (Liong and Subraniam, 1996). The effectiveness of nitrification is influenced by several factors such as : toxic substances; temperature; pH; dissolved oxygen and salinity (Briggs and Turnbull, 1995; Spotte, 1979).

The probiotic formula containing the mixture of selected bacteria, enzymes, and buffer to degrade organic waste (Liong and Subraniam, 1996). Epicin is highly concentrated, stabilized, biological complex containing a blend of genetically selected non-pathogenic, antibiotic resistant (Briggs and Turnbull, 1995). Epicin also contains a natural microbial growth stimulant witch increases the microbial growth, there by increasing the utilization of waste (Briggs and Turnbull, 1995).

Based on the previous investigation was reported by Sarjito *et al*. (1999), it showed that epicin was able to reduce ammona in the culture media at the salinity range of 20–30 ppt. Therefore, it needs a further study about the application of bioaugmentation in order to reduce ammonia in the sediment, to find out the appropriate dose. Therefore, the aim of the research was to investigate both the influence and the effectiveness of bioaugmentation on the reduction of ammonia in sediment of culture medium of the tiger shrimp.

MATERIALS AND METHODS

The research was conducted at hatchery of Fisheries and Marine Science Faculty, Diponegoro University. Bioaugmentation used probiotic "epicin". Epicin is trade mark of probiotic which contains decomposer or nitrification bacteria (Liong and Subraniam, 1996). Sediment was taken from Intensive brockish wish pond of Brackishwater Aquaculture Development Project (BADP), Jepara, Indonesia.

Laboratory scale experiment and split plot randomized design was applied. The treatments was salinity (20; 25 and 30 ppt), as sub-treatment was probiotic concentrations /dose of 0; 0,5; 1,0; and 1,5 ppm and as a group
was day (0; 2; 4 and 6). Three repiclates were applied.

Emulsion of epicin was prepared using method of Briggs and Turnbull (1995). brockish wish pond sediment was taken from intensive culture system and placed on the aquarium for shrimp culture. To investigatethe influence of epicin in order to ammonia waste in the intensive brockish wish pond soil, the study needed 7 days under laboratory conditions. The epicin dose applied (0; 0,5; 1,0; and 1,5 ppm) was only be applied once at beginning the research (an ideal ammonia concentration is about 0,4 ppm). The dose applied was based on the previous experiment conducted (Anonymous, 1996 ; Noor (1996); Sarjito *et al*., 1999).

Ammonia in sediment was analyzed every two days using method of Parsons *et al*. (1989). Daily basis of pH, dissolved oxygen, temperature and salinity were conducted. Anova was used to analyzed the data

RESULT AND DISCUSSION

The result showed that reduction of ammonoiain sediment of aquaculture medium of Tiger shrimp corresponded to epicin dose, so the increasing the dose, followed by high reducingof ammonia in sediment (**Table1.**)

Table 1. The Effect of Bioaugmentation on the Ammonia in the Sediment on the Different Probiotic Epicin Dose and Salinity

Salinity (ppt)	Dose of Epicin (ppm)	Average Ammonia Sediment on The day of (ppm)			
		0	2	4	6
20	0	1.234	1.345	2.435	2.987
	0.5	1.256	0.412	0.726	1.121
	1.0	1.341	0.401	0.704	0.912
	1.5	1.234	0.396	0.669	0.876
25	0	1.286	1.298	2.786	2.435
	0.5	1.231	0.353	0.715	0.972
	1.0	1.256	0.345	0.697	0.867
	1.5	1.261	0.298	0.659	0.812
30	0	1.239	1.875	2.976	2.982
	0.5	1.246	0.217	1.023	1.023
	1.0	1.329	0.229	0.823	0.823
	1.5	1.318	0.214	0.765	0.765

Table 1. Showed that the capability of epicin as a bioaugmentation on reduction of ammoniain the sediment started on the day of 2 in all dosage (0,5 ppm; 1 ppm and 1,5 ppm) and all salinity (20 ppt, 25 ppt and 30 ppt).

However, ammonia tended to increase slightly on the day of 4 and 6. The result was also supported by Anova result that the treatments, especially dose of opican and days of had a observation significant affect on the reduction of ammonia in sediment in media culture of Tiger shrimp ($p<0.01$), with exception of salinity ($p>0.05$). The result indicated that without the treatment applied, ammonia in the sediment increased on the days observation (days of 2, 4 and 6) (**Table 1.)**

The result also showed that the survival rate of Tiger shrimp (*P. monodon*) was at the range of 91,67%–100%. The water quality of the medium culture i.e. temperature (27^{o}C– 28^{o}C); dissolved oxygen (5,8–6,1 ppm); pH (7,8–8,1) and the salinity of 20, 25 and 30 ppt, was suitable for Tiger shrimp.

DISCUSSION

Bioaugmentation was able to reduce ammonia concentration of the sediment. It may be caused by the bacteria of epicin that was able to accimilatize with their environment, and then supported natural bacteria to reduceammonia in the sediment in order to get energy for growth and survival (Apud, 1989; Gaudy and Gaudy, 1981; Spotte, 1979). Besides that, epicin also contains a natural microbial growth stimulant which may increase the natural microbial growth rate, thereby increasing the utilization of ammonia waste in the sediment (Briggs ang Turnbull, 1995). The formulation have also been developed to use for intensive shrimp culture, in order to reduce waste accumulation in both water and sediment of brockish wish pond such as : ammonia, nitrite, nitrate; to reduce uneaten feed in the brockish wish pond sediment; and to increase the water quality(Anonymous, 1996).

Bioaugmentation with dosage treatment was able to decrease the ammonia of the sediment of culture medium. The result was supported by the Anova, where the epicin dose had highly significant affect on the reduction of ammonia in the sediment ($p<0.01$). Dosage influenced the ability of epicin on the ammonia reduction in the water column of culture medium of Tiger shrimp (Sarjito *et al*., 1999). Ammonia in the sediment, in the present investigation, was able to be reduced by epicin with dose of 0,5–1,5 ppm in thevarious salinity (20; 25 and 30 ppt). The same result have also been reported by Sarjito *et al*. (1999) that ammonia in the water was reduced by epicin with dose of 0,5–1,5 ppm, with the best concentration of 1,5 ppm. Present study also found that epicin dose of 1,5 ppm was themost effective dose in the reduction of ammonia in the sediment of culture medium ofTiger shrimp.

The result showed that ammonia reduction may closely relate to the total of decomposer

bacteria in the culture medium of Tiger shrimp. Therefore, the high concentration of epicin was assumed that more total bacteria inoculated and then had a role on the ammonia reduction. The result was in accordance to Sarjito's *et al*., (1999) founding that the most effective dose of epicin to reduce ammonia in the water was 1,5 ppm. The dose was higher compared to other study i.e. : 1,0 ppm (Anonymous 1996).

The result also indicate that decomposer bacteria (contain of epicin) as bioaugmentation showed the ability to reduce ammonia in the sediment on the day of 2, although increasing sediment ammonia still occurred on the day of 4 and 6. However, the concentration of ammonia in the sediment was still lower compared to the concentration of ammonia in the sediment at the beginning of the experiment (day 0). Least Significance Different Test also showed that ammonia concentration of the day of 6 was high significant different ($p<0.01$) compared to ammonia concentration in the sediment of the day of 0, 2 and 4; between the day of 0 compared to the day of 2 and 4; between the day of 4 and the day of 2. In the contrary, Sarjito *et al.* (1999) found that on the days of 4 and 6, water ammonia had increased, in turn, there was no difference between the two of days. It may correlate to the availability of the sediment as substrate for the growth of natural decomposer bacteria i.e : *Nitrosomonas* sp. and *Nitrobacter* sp. (Forteath, 1988; Gaudy and Gaudy, 1981; Spotte, 1979). and also epicin bacteria i.e : *Basillus*; *Nitrosomonas*; and *Nitrobacter*[11)]; *Bacillus subtillis*; *B. lychenniformis*, *B. megatorium*, *Lactobacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp. and *Saccharomyces cereviceae* (Noor, 1996) that have a role on the sediment ammonia reduction in the sediment. For growth and multiply, suitable substrate and environment were needed by bacteria (Forteath, 1988; Gaudy and Gaudy, 1981; Santoso *et al*., 1996; Spotte, 1979). Besides that, nitrification bacteria in the sediment were 1000 times more compared to the contain nitrification bacteria suspended in the water (Spotte, 1979), as this present experiment detected the lower concentration of ammonia in the water. Increasing ammonia in the sediment on the days of 4 and 6 may due to the bacteria selection occurred naturally. Dramatic ammonia reduction in the sediment occurred on the day of 2, resulted the limitation of ammonia, as source of bacterial energy, therefore only certain bacteria selected and not all the bacteria were able to survive. As the result, the total bacteria population may decrease on the days of 4 or 6, therefore increase the ammonia in the sediment. Besides that, high survival rate of Tiger shrimp may contribute on the ammonia excretion(Claybrook, 1993; Wickins, 1976).

Salinity affected on the bioaugmentation process (Sarjito *et al*., 1999). In contrast, the present experiment found that salinity did not have significant affect on the ammonia reduction in the sediment ($p>0.05$). The similar finding was also reported that epicin as a biouagmentation material works effectively in salinity of 0–40 ppt (Anonymous, 1996). High survival rate (91,27–100%) may due to the high water quality of culture medium of Tiger shrimps during the experiment (Apud, 1989; Boyd, 1982; Chamberlain, 1990; Maguire and Allan, 1990). Ammonia concentration in the sediment of 2,088 ppm, was able to be tolerated by Tiger shrimp. The toxicity of ammonia depends on the unionized ammonia 6, 8, 19 ; pH; temperature and salinity (Boyd, 1989; Colt and Amstrong, 1991; Emerson *et al*., 1975; Santoso *et al*.,1996; Spotte, 1979).

In conclusion, this present experiment indicated that bioaugmentation using probiotic epicin was able to reduce ammonia in the sediment of culture medium of Tiger shrimp. Although salinity did not affect on the bioaugmentation work, however the dose and day of observation did affect significantly on the reduction of ammonia in the sediment. It was also determined that the most effective dose of bioaugmentation using probiotic epicin was 1,5 ppm.

ACKNOWLEDGMENT

We would like to gratitude and thank to Director of Research and Community Service Development, Director General Higher Education for their funding; we would like to honor to unknown reviewer for his corrections and suggestions. Special thank to Ir. Agung Setiarto, MSc. For his valuable suggestions to improve the manuscript. We are also indebted to Rita T. for preparing her manuscript.

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Manuscript decision

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Thank you for submitting you work to Journal of Coastal Development.

Yours sincerely

Prof Ocky Karna Radjasa
Editor-in-Chief

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APPLICATION OF BIOAUGMENTATION TO SOLVE AMMONIA IN THE SEDIMENT OF THE CULTURE MEDIUM OF TIGER SHRIMP (*Penaeus monodon* F.) IN DIFFERENT SALINITIES

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ABSTRACT

Bioaugmentation was used to solve the ammonia problem, because this method is relatively safe to the environment. The objective of this research was to determine the influence and effectiveness of bioaugmentation on the reduction of ammonia in the sediment in culture medium of Tiger shrimp (P. monodon). Laboratory scale experiment and splitted plot randomized design was performed. The main treatment was probiotic epicin concentration of 0; 0.5; 1.0; and 1 ppm; as sub-treatment was the salinity (20; 25 and 30 ppt), and as a group was dayobservation (0; 2; 4 and 6). Sediment from intensive culture system was taken from brackish water pond of Brackishwater Aquaculture Development Project (BADP), Jepara. Sediment ammonia was analyzed using methods of Parson et al.,(1989). The research was conducted at hatchery of Fisheries and Marine Science Faculty of Diponegoro University, Teluk Awur, Jepara. The result indicated that bioaugmentation was able to reduce ammonia concentration in the sediment. Epicin has high significantly effected on the reduction of ammonia in the sediment ($p<0.01$). The capability of epicin as a bioaugmentation started on the day of 2. Therefore, the results also showed that a time (day) was highly significant affect on the reduction of ammonia in the sediment ($p<0.01$). However, salinity did not affect on the reduction of ammonia in the sediment ($p>0.05$). The most effective epicin dose to reduce ammonia in the sediment of the culture medium of Tiger shrimp (P. monodon) was 1.5 ppm.

Key words: Bioaugmentation, Ammonia, Epicin, Sediment, Salinity

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INTRODUCTION

The problem of high pollutant concentration such as uneaten feed and waste products in sediment has been frequently faced at the penaid shrimp (*Penaus monodon* F.) culture and, in turn, it may reduce the water quality (Maguire and Allan, 1990). The increasing of pollutant or toxic substance in both sediment and water lead to a limitatian of the life process and in certain condition, it may increase the shrimp mortality (Apud, 1989; Maguire and Allan, 1990). Therefore, toxic substance, including ammonia, has caused the main failure of the culture (Maguire and Allan, 1990) and one of limiting factors in the intensive aquaculture system (Forteath, 1988).

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Laboratory scale experiment and split plot randomized design was applied. The treatments was salinity (20; 25 and 30 ppt), as sub-treatment was probiotic concentrations / dose of 0; 0,5; 1,0; and 1,5 ppm and as a group was day (0; 2; 4 and 6). Three repiclates were applied.

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Ammonia in sediment was analyzed every two days using method of Parsons *et al*. (1989). Daily basis of pH, dissolved oxygen, temperature and salinity were conducted. Anova was used to analyzed the data.

RESULT AND DISCUSSION

The result showed that reduction of ammonoia in sediment of aquaculture medium of Tiger shrimp corresponded to epicin dose, so the increasing the dose, followed by high reducing of ammonia in sediment (**Table1.**)

Table 1. The Effect of Bioaugmentation on the Ammonia in the Sediment on the Different Probiotic Epicin Dose and Salinity

Salinity (ppt)	Dose of Epicin (ppm)	Average Ammonia Sediment on The day of (ppm)			
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Table 1. Showed that the capability of epicin as a bioaugmentation on reduction of ammonia in the sediment started on the day of 2 in all dosage (0,5 ppm; 1 ppm and 1,5 ppm) and all salinity (20 ppt, 25 ppt and 30 ppt). However, ammonia tended to increase slightly on the day of 4 and 6. The result was also supported by Anova result that the treatments, especially dose of opican and days of had a observation significant affect on the reduction of ammonia in sediment in media culture of Tiger shrimp ($p<0.01$), with exception of salinity ($p>0.05$). The result indicated that without the treatment applied, ammonia in the sediment increased on the days observation (days of 2, 4 and 6) (**Table 1.)**

The result also showed that the survival rate of Tiger shrimp (*P. monodon*) was at the range of 91,67%–100%. The water quality of the medium culture i.e. temperature (27^oC–28^oC); dissolved oxygen (5,8–6,1 ppm); pH (7,8–8,1) and the salinity of 20, 25 and 30 ppt, was suitable for Tiger shrimp.

Discussion

Bioaugmentation was able to reduce ammonia concentration of the sediment. It may be caused by the bacteria of epicin that was able

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to accimilatize with their environment, and then supported natural bacteria to reduce ammonia in the sediment in order to get energy for growth and survival (Apud, 1989; Gaudy and Gaudy, 1981; Spotte, 1979). Besides that, epicin also contains a natural microbial growth stimulant which may increase the natural microbial growth rate, thereby increasing the utilization of ammonia waste in the sediment (Briggs ang Turnbull, 1995). The formulation have also been developed to use for intensive shrimp culture, in order to reduce waste accumulation in both water and sediment of brockish wish pond such as : ammonia, nitrite, nitrate; to reduce uneaten feed in the brockish wish pond sediment; and to increase the water quality (Anonymous, 1996).

Bioaugmentation with dosage treatment was able to decrease the ammonia of the sediment of culture medium. The result was supported by the Anova, where the epicin dose had highly significant affect on the reduction of ammonia in the sediment ($p<0.01$). Dosage influenced the ability of epicin on the ammonia reduction in the water column of culture medium of Tiger shrimp (Sarjito *et al*., 1999). Ammonia in the sediment, in the present investigation, was able to be reduced by epicin with dose of 0,5–1,5 ppm in the various salinity (20; 25 and 30 ppt). The same result have also been reported by Sarjito *et al*. (1999) that ammonia in the water was reduced by epicin with dose of 0,5–1,5 ppm, with the best concentration of 1,5 ppm. Present study also found that epicin dose of 1,5 ppm was the most effective dose in the reduction of ammonia in the sediment of culture medium of Tiger shrimp.

The result showed that ammonia reduction may closely relate to the total of decomposer bacteria in the culture medium of Tiger shrimp. Therefore, the high concentration of epicin was assumed that more total bacteria inoculated and then had a role on the ammonia reduction. The result was in accordance to Sarjito's *et al*., (1999) founding that the most effective dose of epicin to reduce ammonia in the water was 1,5 ppm. The dose was higher compared to other study i.e. : 1,0 ppm (Anonymous 1996).

The result also indicate that decomposer bacteria (contain of epicin) as bioaugmentation showed the ability to reduce ammonia in the sediment on the day of 2, although increasing sediment ammonia still occurred on the day of 4 and 6. However, the concentration of ammonia in the sediment was still lower compared to the concentration of ammonia in the sediment at the beginning of the experiment (day 0). Least Significance Different Test also showed that ammonia concentration of the day of 6 was high significant different ($p<0.01$) compared to ammonia concentration in the sediment of the day of 0, 2 and 4; between the day of 0 compared to the day of 2 and 4; between the day of 4 and the day of 2. In the contrary, Sarjito *et al.* (1999) found that on the days of 4 and 6, water ammonia had increased, in turn, there was no difference between the two of days. It may correlate to the availability of the sediment as substrate for the growth of natural decomposer bacteria i.e : *Nitrosomonas* sp. and *Nitrobacter* sp. (Forteath, 1988; Gaudy and Gaudy, 1981; Spotte, 1979). and also epicin bacteria i.e : *Basillus*; *Nitrosomonas*; and *Nitrobacter*[11)]; *Bacillus subtillis*; *B. lychenniformis*, *B. megatorium*, *Lactobacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp. and *Saccharomyces cereviceae* (Noor, 1996) that have a role on the sediment ammonia reduction in the sediment. For growth and multiply, suitable substrate and environment were needed by bacteria (Forteath, 1988; Gaudy and Gaudy, 1981; Santoso *et al*., 1996; Spotte, 1979). Besides that, nitrification bacteria in the sediment were 1000 times more compared to the contain nitrification bacteria suspended in the water (Spotte, 1979), as this present experiment detected the lower concentration of ammonia in the water. Increasing ammonia in the sediment on the days of 4 and 6 may due to the bacteria

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selection occurred naturally. Dramatic ammonia reduction in the sediment occurred on the day of 2, resulted the limitation of ammonia, as source of bacterial energy, therefore only certain bacteria selected and not all the bacteria were able to survive. As the result, the total bacteria population may decrease on the days of 4 or 6, therefore increase the ammonia in the sediment. Besides that, high survival rate of Tiger shrimp may contribute on the ammonia excretion (Claybrook, 1993; Wickins, 1976).

Salinity affected on the bioaugmentation process (Sarjito *et al*., 1999). In contrast, the present experiment found that salinity did not have significant affect on the ammonia reduction in the sediment ($p>0.05$). The similar finding was also reported that epicin as a biouagmentation material works effectively in salinity of 0–40 ppt (Anonymous, 1996). High survival rate (91,27–100%) may due to the high water quality of culture medium of Tiger shrimps during the experiment (Apud, 1989; Boyd, 1982; Chamberlain, 1990; Maguire and Allan, 1990). Ammonia concentration in the sediment of 2,088 ppm, was able to be tolerated by Tiger shrimp. The toxicity of ammonia depends on the unionized ammonia 6, 8, 19 ; pH; temperature and salinity (Boyd, 1989; Colt and Amstrong, 1991; Emerson *et al*., 1975; Santoso *et al*., 1996; Spotte, 1979).

In conclusion, this present experiment indicated that bioaugmentation using probiotic epicin was able to reduce ammonia in the sediment of culture medium of Tiger shrimp. Although salinity did not affect on the bioaugmentation work, however the dose and day of observation did affect significantly on the reduction of ammonia in the sediment. It was also determined that the most effective dose of bioaugmentation using probiotic epicin was 1,5 ppm.

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